Analysis of PCBs, Pesticides, and PAHs in Air and Precipitation Samples:

IADN Project

Sample Preparation Procedure

Ilora Basu
School of Public and Environmental Affairs
Indiana University
Bloomington, IN 47405

June 1995

Version 1.0

Analysis of PCBs, Pesticides, and PAHs in Air and Precipitation Samples: IADN Project - Sample Preparation Procedure

1.0 Summary

This guide is written for chemists and technical assistants so that everybody follows the same details in preparation of IADN samples. We process Air (both vapor and particle phase) and Precipitation samples for analysis of PCBs, pesticides and PAHs. A brief description of the 12 sections covered in this volume of SOP follows:

1.1 Section I: Cleaning

This section describes the procedures of soap and water cleaning, muffling, and ultrasonic cleaning of glassware and other tools.

1.2 Section II: Precleaning

This section covers the long procedure of precleaning XAD_2 with different types of solvents. The procedure was originally standardized by Steve Eisenreich and later on it was modified in our laboratory. Besides XAD_2, we have also described the precleaning procedure of glass wool, silica gel, boiling chips, and glass fibre filters.

1.3 Sections III and IV: Extraction

Soxhlet extraction of air vapor, air particle and precipitation samples from XAD_2 cartridges and GFF are described in these two sections. Detailed procedures for concentration by rotary evaporation, solvent exchange and back extraction are documented. We also mention QC samples and spiking samples with recovery standard etc.

1.4 Section V: Silica Column Chromatography

After extraction, the extracts are cleaned up from interfering compounds and fractionated into three different fractions through silica gel deactivated to 4%. First fraction is collected with Hexane which contains all PCBs and pesticides like HCB and DDE. The second fraction which is collected with 50% CH_2Cl_2 in Hexane contains all PAHs and pesticides like á and ã HCHs, Dieldrin, DDD, DDT, ã Chlordane, á Chlordane, and T-Nonachlor. The third fraction is collected in Methanol and contains atrazine.

1.5 Sections VI and VII: Transfer and Nitrogen Blow Down

These two sections describe the procedure for final transfer of prepared samples from flasks to 4 mL amber vials. The samples are then concentrated to desired volume by a slow stream of ultra-pure nitrogen. Final volumes are adjusted depending on types of samples and time of collection to ensure GC chromatograms are not off scale.

1.6 Sections VIII and IX: Spiking and Making Microvials

After proper concentration by Nitrogen blow down, each sample is spiked with known amount of internal standard or quantitation standard. Subsamples are then transferred to autosampler microvials for GC analysis.

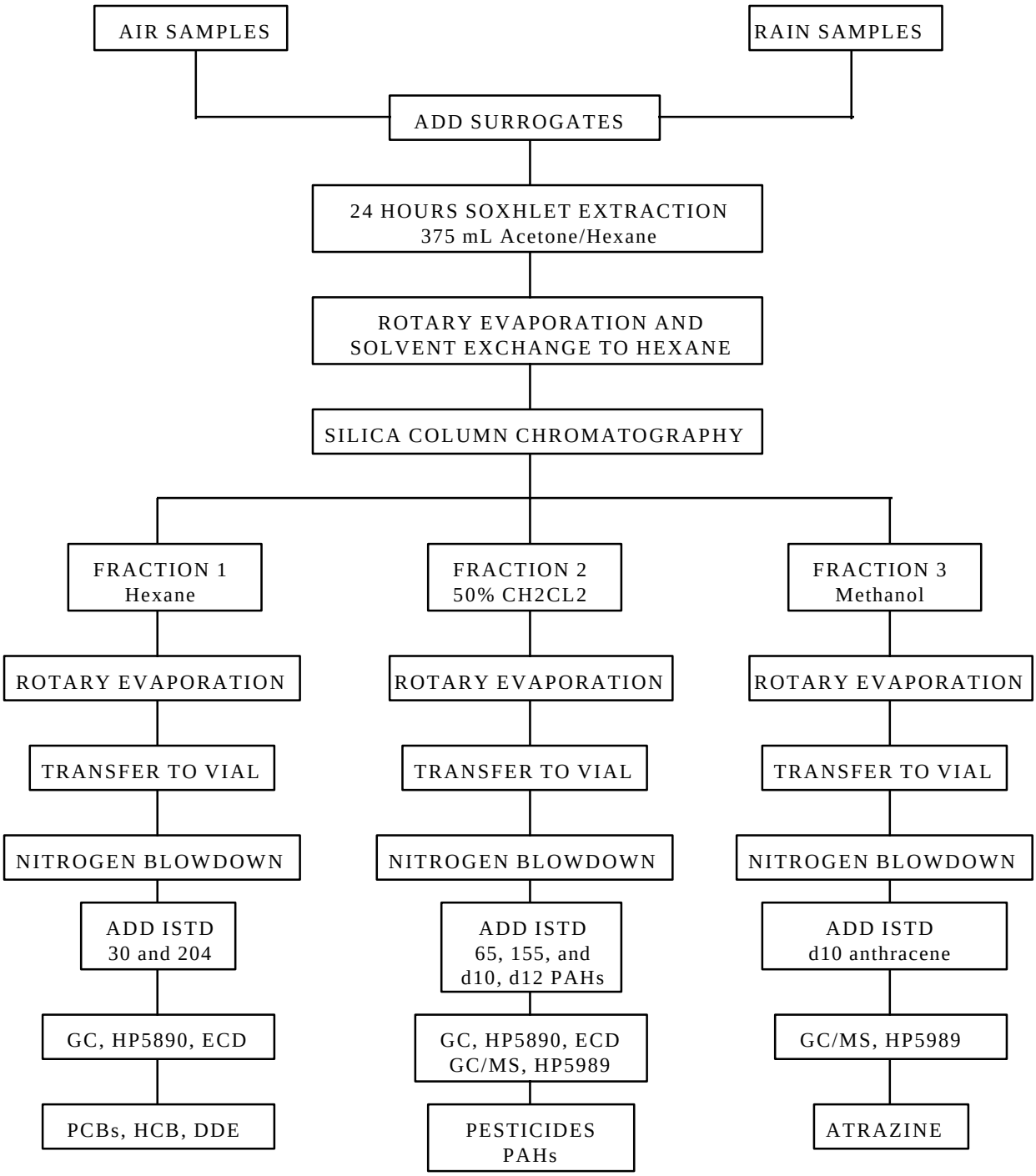
1.7 Section X: Standards

Procedures for preparation of all stock standards, working standards, calibration standards, recovery standards, and spiking internal standards are compiled in this section.

1.8 Section XI: Safety

Some of the safety rules that we follow for day to day laboratory work are mentioned here. Procedure for waste disposal is also included in this section.

2.0 The Flow Chart of Sample Preparation



3.0 Cleaning

3.1 Glassware

3.1.1 Supplies

3.1.1.1 *Glassware:*

Assemble what is to be cleaned

3.1.1.2 *Non-glassware:*

Micro cleaning solution
DI water
Dish washing brush

3.1.1.3 *Equipment:*

Drying oven
Muffle furnace
Acid bath: 50/50 H_2SO_4 and HNO_3

3.1.2 Procedures

1) Wash/Dry
   Wash glassware thoroughly with soap and water. Use brush if necessary.
   Glassware with bad stains should be rinsed with MeOH or CH_2Cl_2 before using the soap and water procedure. If still not clean, soak in acid bath overnight, then wash thoroughly with soap and water.
   Volumetric pipettes used for standards **must** soak in acid bath overnight.
   Rinse glassware thoroughly with tap water.
   Rinse glassware thoroughly with DI water.
   Dry glassware in air.
   Cover all open ends with foil.
2) Muffle glassware at 450°C for four hours. If glassware is not clean after muffling at 450°C for four hours, muffle at 500°C for four hours.
3) Allow glassware to cool to 100°C before removing from furnace.
4) Store.

3.1.3 Comment

Always use dull side of foil towards glassware.

3.2 Stainless Steel Tools

3.2.1 Supplies

3.2.1.1 *Glassware:*
-none-

3.2.1.2 *Non-glassware:*

Items to be cleaned: forceps, spatula, scissors, etc.
CH_2Cl_2 in teflon bottle
Cl^- waste bottle

3.2.1.3 *Equipment:*

drying oven

3.2.2 Procedures

1) Wash with soap and water.
2) Rinse well with tap water.
3) Rinse thoroughly with DI water.
4) Dry at room temperature overnight.
5) Wrap each tool separately in foil.
6) Store.

3.2.3 Comment

ALWAYS rinse with CH_2Cl_2 before use.

3.3 Amber Glass Vials and Pasteur Pipettes

3.3.1 Supplies

3.3.1.1 *Glassware:*

400 mL beaker

3.3.1.2 *Non-glassware:*

Foil

3.3.1.3 *Equipment:*

Muffle furnace

3.3.2 Procedure

1) Wrap glass in foil or place in beaker and cover beaker with foil.
2) Muffle at 450°C for four hours.
3) Cool to 100°C; remove from oven.
4) Insert teflon liner into vial cap and cap the vial as soon as the vial comes out of the

oven.

5) Store in a beaker covered with foil.

3.4 Teflon liners

3.4.1. Supplies

3.4.1.1 *Glassware:*

400 mL beaker

3.4.1.2 *Non-glassware:*

Foil
CH_2Cl_2
Cl^- waste bottles

3.4.1.3 *Equipment:*

Ultrasonicator

3.4.2 Procedures

1) Place teflon liners in glass beaker; cover with CH_2Cl_2.
2) Ultra-sonicate for 15 minutes. Drain CH_2Cl_2.
3) Repeat.
4) Place in 70°C drying oven for two hours.
5) Store in sealed jar.

3.5 Micropipette Tubes, GC Microvials, and Stainless N_2 Blowdown Needles

3.5.1 Supplies

3.5.1.1 *Glassware:*

400 mL or larger beaker

3.5.1.2 *Non-glassware:*

CH_2Cl_2
Cl^- waste bottle

3.5.1.3 *Equipment:*

Muffle furnace

3.5.2 Procedures

1) Micropipette tubes
Before using, rinse with CH_2Cl_2 and air dry.
2) GC microvials
Place microvials, open end up, in a clean beaker. Cover vials with CH_2Cl_2, making sure **NO** air bubbles remain in the microvials. Cover loosely with foil.
Sonicate microvials for 10 minutes.
Drain solvent, and repeat twice more. (The microvials should be sonicated a total of three times.)
Drain all solvent and transfer microvials to clean beaker; cover with foil. Muffle at 450°C for four hours. After furnace returns to 100°C (or the next morning) remove vials from furnace.
Store in sealed container.
3) Stainless N_2 blowdown needles
Place needles in a clean beaker and cover with CH_2Cl_2. Cover loosely with foil.
Sonicate needles for 10 minutes.
Drain solvent, and repeat twice more. (The needles should be sonicated a total of three times.)
Drain all solvent and transfer needles to clean beaker. Cover beaker with foil.
Label beaker "CLEAN"; store near the N_2 blowdown unit.

3.6 Teflon Stopcocks and Lids for Sample Jars

3.6.1 Supplies

3.6.1.1 *Glassware:*

-none-

3.6.1.2 *Non-glassware:*

Alconox
DI water
kimwipes

3.6.1.3 *Equipment*

-none-

3.6.2 Procedure

1) Wash stopcocks and lids with Alconox and tap water.
2) Rinse stopcocks and lids with DI water.
3) Air dry on kimwipes.
4) Storage
Store the stopcocks in muffled jar or beaker covered with foil.

Place lids on muffled sample jars or wrap them in foil.

4.0 Precleaning

4.1 Glass Wool

4.1.1 Supplies

4.1.1.1 *Glassware:*

Sample jar and lid
Glasswool

4.1.1.2 *Non-glassware:*

Foil

4.1.1.3 *Equipment:*

Scissors
Muffle furnace

4.1.2 Procedures

1) Cut glass wool into 2" pieces.
2) Put into muffled glass sample jar; cover jar with foil.
3) Muffle at 450°C for four hours.
4) Screw lid on jar (do not remove foil).
5) Store.

4.2 Teflon Boiling Chips

4.2.1 Supplies

4.2.1.1 *Glassware:*

Soxhlet extractor: extra large (71/60 and 29/42 joints)
large (55/50 and 24/40 joints)
Condenser: 71/60 joint for extra large soxhlet
55/50 joint for large soxhlet
Round bottom flask: 1 liter for extra large soxhlet
500 ml for large soxhlet
Adaptor (for extra large soxhlet, converts 29/42 joint to 24/40 joint)
Sample jar and lid
1 L beaker

4.2.1.2 *Non-glassware:*

Boiling chips

CH_2Cl_2
CH_2Cl_2 in squirt bottle
Methanol in squirt bottle
Cl^- solvent waste container
Non-Cl^- solvent waste container
Cellulose thimbles: 60 x 180 mm for extra large soxhlet
43 x 123 mm for large soxhlet
Foil
Cork ring for round bottom flask

4.2.1.3 *Equipment:*

Variable autotransformer (aka variac)
Heating mantle for either 1 L or 500 mL round bottom flask
Drying oven

4.2.2. Procedures

Day 1

1) Thoroughly rinse inside of condenser and outside of joint with solvent in squirt bottles: first with methanol, then with CH_2Cl_2. Cover joint and exhaust tube with foil.
2) Add five or six boiling chips to flask. Add appropriate amount of CH_2Cl_2 to flask.
3) Place <u>new</u> teflon boiling chips in appropriate cellulose thimble. Place thimble in soxhlet extractor.

	Thimble Size	Flask Size (mL)	CH_2Cl_2 (mL)
large soxhlet	43 x 123	500	300
extra large soxhlet	60 x 180	1000	600

4) Assemble flask/soxhlet/condenser/adaptor (if necessary) apparatus.
5) Turn on heater to give proper boiling (set variac to 40-45).
6) Turn on chilled water for condenser.
7) Extract for 18 to 24 hours.

Day 2

1) Turn heat off; let cool 15 to 30 minutes.
2) Turn off condenser water.
3) Drain as much solvent from soxhlet as possible.

4) Remove thimble from soxhlet, place upside down in a 1 L beaker, cover loosely with foil.

5) Place boiling chips in a 70°C oven: Every 10 to 15 minutes, check boiling chips, shaking beaker to determine if all solvent has evaporated. Let boiling chips remain in oven two to four hours, until dry.
*****WARNING: BEWARE OF SOLVENT FUMES.*****
6) Wrap thimble in foil and store for future use.
7) Place in boiling chips in clean sample jar; cover with foil and lid.
8) Store on shelf.

Note: Boiling chips can be directly placed in soxhlet plugged with glasswool instead of using cellulose thimble.

4.3 Sodium Sulfate (Na_2SO_4)

4.3.1 Supplies

4.3.1.1 *Glassware:*

500 mL beaker
Sample jar and lid

4.3.1.2 *Non-glassware:*

Sodium sulfate (Na_2SO_4)
Foil

4.3.1.3 *Equipment:*

Muffle oven
Drying oven
Desiccator

4.3.2 Procedures

1) New Na_2SO_4
Put Na_2SO_4 in a clean muffled beaker and bake at 450°C for four hours or overnight.
Cool to 100°C in oven. Remove.
Place in clean sample jar; cover with foil and lid.
Store in desiccator.
2) Reconditioning Na_2SO_4 (to be done every two weeks):
Place Na_2SO_4 in 100°C drying oven overnight.
Remove from oven; cover with foil and lid.
Store in desiccator.

4.4 XAD_2

4.4.1 Supplies

4.4.1.1 *Glassware:*

Soxhlet extractor and condenser 71/60 and 29/42 joints
Six 1 L round bottom flasks with 24/40 joint
Six glass stoppers (24/40 joint)
One 1 L beaker
Two 400 mL beakers (one need not be clean)
Adaptor to convert 29/42 to 24/40

4.4.1.2 *Non-glassware:*

Boiling chips
CH_2Cl_2
Hexane
Methanol
Acetone
HPLC grade water: EM Science
CH_2Cl_2 in squirt bottle
Methanol in squirt bottle
Cl^- solvent waste container
Non-Cl^- solvent waste container
Foil
Glass wool
Six cork rings

4.4.1.3 *Equipment:*

Heating mantle for 1 L flask
Variable autotransformer (aka variac)
Refrigerator or freezer

4.4.2 Procedures for dry XAD_2 for air sample cartridges

Day 1

1) Place XAD_2 in extractor plugged with glass wool.
2) Rinse XAD_2 with tap water many times, stirring to remove foam and small particles. Use kimwipes to remove foam.
3) Rinse with small amount of methanol three times to remove water.
4) Add 500 mL of methanol to 1 L flask.
5) Add about 20 boiling chips to flask.
6) Assemble flask/soxhlet/condenser apparatus.
7) Turn on heater to give proper boiling (set variac to 60-65 for methanol).
8) Turn on chilled water for condenser.

9) Cover soxhlet and flask with foil.
10) Extract with methanol for 24 hours.

Day 2

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much methanol from soxhlet as possible.
3) Add 500 mL acetone to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 45 for acetone).
6) Cover soxhlet and flask with foil.
7) Extract with acetone for 24 hours.

Day 3

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much acetone from soxhlet as possible.
3) Add 500 mL hexane to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 40-45 for hexane).
6) Cover soxhlet and flask with foil.
7) Extract with hexane for 24 hours.

Day 4

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much hexane from soxhlet as possible.
3) Add 500 mL CH_2Cl_2 to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 40-50 for CH_2Cl_2).
6) Cover soxhlet and flask with foil.
7) Extract with Ch_2Cl_2 for 24 hours.

Day 5

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much CH_2Cl_2 from soxhlet as possible. Wait 15 minutes. Drain as much solvent as possible.
3) Add 100 mL hexane to the soxhlet. Wait 15 minutes, then hand flush. Repeat at least three times, until the level of the solvent in the siphon tube is the same as in the soxhlet.
4) Add 500 mL hexane to 1 L flask.
5) Add about 20 boiling chips to flask.
6) Turn on heater (set variac to 40-45 for hexane).
7) Cover soxhlet and flask with foil.
8) Extract with hexane for 24 hours. Flushing may need to be induced twice before it flushes on its own.

Day 6

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much hexane from soxhlet as possible.
3) Add 500 mL 50% acetone/50% hexane to 3 L flask.
4) Add boiling chips to flask.

5) Turn on heater (set variac to 40-45 for acetone/hexane).
6) Cover soxhlet and flask with foil.
7) Extract with acetone/hexane for 24 hours.

Day 7

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much acetone/hexane from soxhlet as possible.
3) Pour XAD_2 in a beaker and dry overnight in 65°C oven.
4) Store in amber bottle in freezer at -20°C for up to three months.
5) Keep subsample in separate jar for checking lab blank and matrix spike.

4.4.3 Procedures for wet XAD_2 for precipitation sample cartridges

Day 1

1) Place XAD_2 in soxhlet plugged with glass wool.
2) Rinse XAD_2 with water many times, stirring to remove foam and small particles. Use kimwipes to remove foam.
3) Rinse with small amount of methanol three times to remove water.
4) Add 500 mL methanol to 1 L flask.
5) Add about 20 boiling chips to flask.
6) Assemble flask/soxhlet/condenser apparatus.
7) Turn on heater to give proper boiling (set variac at 60-65 for methanol).
8) Turn on chilled water for condenser.
9) Cover soxhlet and flask with foil.
10) Extract for 24 hours.

Day 2

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much methanol from soxhlet as possible.
3) Add 500 mL acetone to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 40-45 for acetone).
6) Cover soxhlet and flask with foil.
7) Extract with acetone for 24 hours.

Day 3

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much acetone from soxhlet as possible.
3) Add 500 mL hexane to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 40-45 for hexane).
6) Cover soxhlet and flask with foil.
7) Extract with hexane for 24 hours.

Day 4

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much hexane from soxhlet as possible.
3) Add 500 mL CH_2Cl_2 to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 40 for CH_2Cl_2).

6) Cover soxhlet and flask with foil.
7) Extract with CH_2Cl_2 for 24 hours.

Day 5

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much CH_2Cl_2 from soxhlet as possible. Wait 15 minutes. Drain as much solvent as possible.
3) Add 100 mL hexane mixture to the soxhlet. Wait 15 minutes, then drain solvent . Repeat at least three more times, until level of solvent in the siphon tube is the same as in the soxhlet.
4) Add 500 mL hexane to 1 L flask.
5) Add about 20 boiling chips to flask.
6) Turn on heater (set variac at 40-45 for hexane).
7) Cover soxhlet and flask with foil.
8) Extract with hexane for 24 hours.

Day 6

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much hexane from soxhlet as possible.
3) Add 500 mL acetone to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac at 40-45 for acetone).
6) Cover soxhlet and flask with foil.
7) Extract with acetone for 24 hours.

Day 7

1) Turn off heater; cool 15 to 30 minutes.
2) Flush as much acetone from soxhlet as possible.
3) Add 500 mL methanol to 1 L flask.
4) Add about 20 boiling chips to flask.
5) Turn on heater (set variac to 60-65 for methanol).
6) Cover soxhlet and flask with foil.
7) Extract with methanol for 24 hours.

Day 8 or so

1) Turn off heater; cool 15 to 30 minutes.
2) Turn off condenser water.
3) Flush as much methanol from soxhlet as possible.
4) Rinse XAD_2 at least three times with EM Science HPLC grade water (until XAD_2 does not smell of methanol).
5) Store the clean XAD_2 in DI water in amber bottle in the refrigerator at 4°C. (The resin may be stored in this manner for up to three months.)

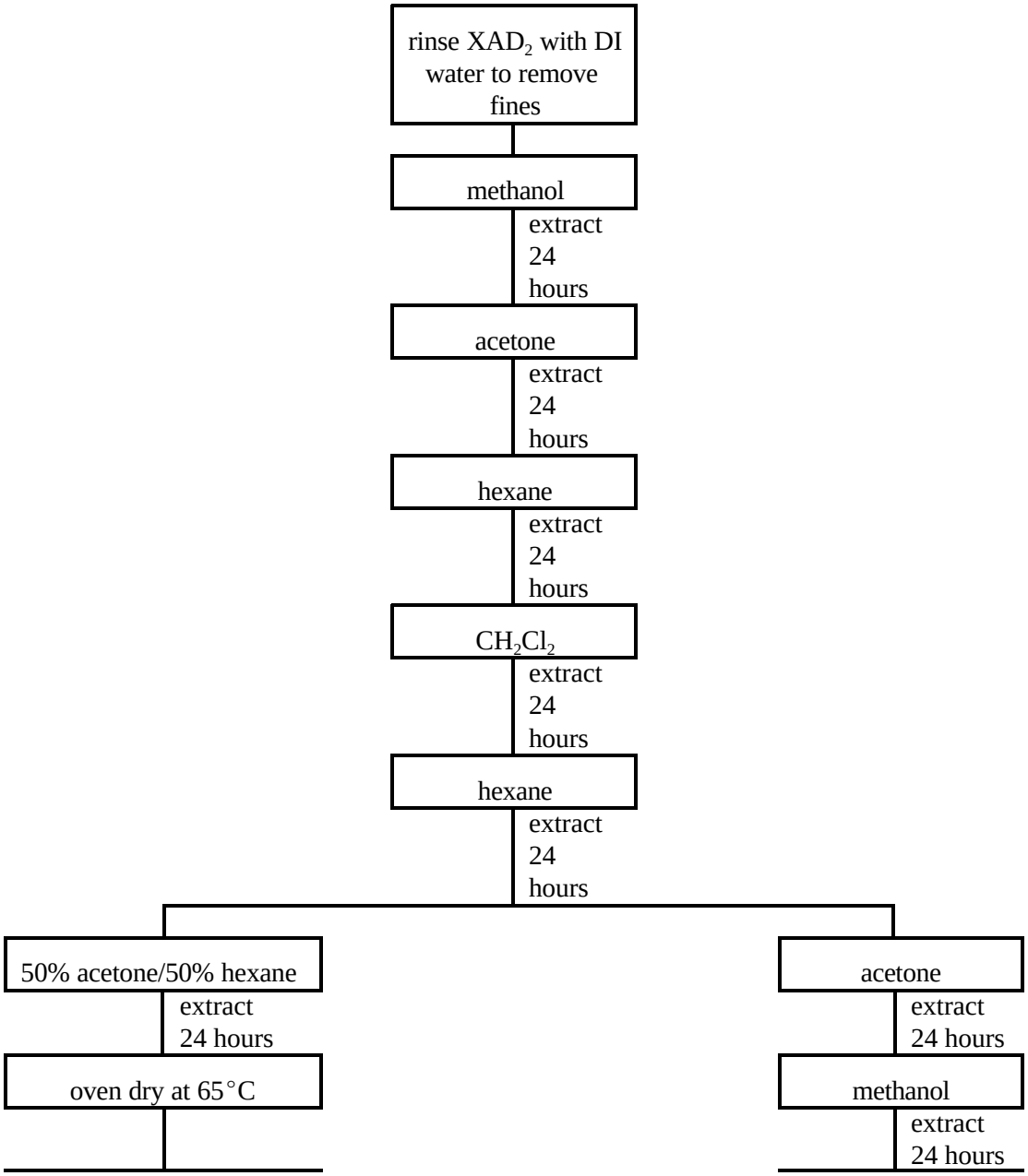
4.4.4 Comments

4.4.4.1 Variac settings may vary from autotransformer to autotransformer. Check that the solvent is boiling properly (nice rolling boil).

4.4.4.2 If XAD_2 is re-used after sample extraction, it is not necessary to rinse with DI water before extracting. The cleaning process can begin by extracting with methanol.

4.4.4.3 Sometime solvent does not siphon very well. Induce siphoning by hand as many times as possible. Allow extra time in case of improper flushing.

4.4.5 Flowchart of XAD_2 precleaning procedure



store at -20°C in amber bottle	exchange to HPLC water; store at 4°C in amber bottles
AIR SAMPLES	PRECIPITATION SAMPLES

4.5 Silica and Quartz Fiber Filters (QF)

4.5.1 Silica

It has been determined silica is adequately cleaned during the activation process therefore no additional processing is necessary.

4.5.2 Quartz fiber filters (QF)

Each QF is wrapped up by aluminum foil separately and then muffled to 450°C for four hours. After it reaches ambient temperature, about 25 are wrapped again in aluminum foil and stored in freezer in a sealed plastic bag.

5.0 Air Samples, Particle and Vapor Phase: QF and XAD_2 Cartridges

5.1 Extraction

5.1.1 Supplies

5.1.1.1 *Glassware:*

Large soxhlet extractor (55/50 and 24/40 joints)
Condenser (55/50 joint)
500 mL round bottom flask (24/40 joint)
Glass stopper (24/40 joint)
400 mL beaker
Micro-dispenser (50 or 100 µL) and 1 mL pipette

5.1.1.2 *Non-glassware:*

Boiling chips
Acetone
Hexane
Spiking standards:

Standard	Concentration
PCB surrogate standard	Congener 14: 200 ng/mL
	Congener 65: 50 ng/mL

	Congener 166: 50 ng/mL
pesticide recovery standard	100 ng of each pesticide/mL
PAH recovery standard	2 µg of each PAH/mL
Dibutylchlorendate	500 ng/mL
Terbutylazine	5600 ng/mL
Atrazine	2000 ng/mL
d_{10} Phenanthrene	2 µg/mL
PCB recovery standard	683 ng of PCBs/mL

CH_2Cl_2 in squirt bottle
Methanol in squirt bottle
Cl^- solvent waste bottle
Non-Cl^- solvent waste bottle
Cork ring (size #2)
Glasswool
12" rod (glass or metal)
Large tweezers
Small tweezers
Foil
Scissors

5.1.1.3 *Equipment:*

Heating mantle and variable autotransformer or multi-unit extraction heater

5.1.2 Procedures

One set of sample is extracted in two days. The set includes 10-12 samples (including one duplicate), one field blank, one lab blank, and one combination matrix spike. In combination matrix spike, the matrix is spiked with known amount of PCBs, Pesticides,PAHs, and atrazine to calculate recovery of each compound. A name will be assigned to each set on the day of extraction (month, year and type of sample), such as S94C, in which:

S = Month of sample collection, such as September
94 = year of sample collection
C = Type of sample, such as cartridge

Day 1

1) Remove spiking standards from freezer. Standards **must** be at ambient temperature before using. (Ambient temperature is achieved in about two hours.)
   surrogate PCB standard- PCB #14, 65, 166
   surrogate pesticide standard- dibutylchlorendate
   surrogate atrazine standard- trbutylazine
   surrogate PAH standard- d_{10} phenenthrene
   pesticide recovery standard
   PAH recovery standard
   PCB recovery standard
   Atrazine recovery standard
2) Thoroughly rinse inside of condenser and outside of joint with solvent in squirt bottles: first with methanol, then with CH_2Cl_2. Cover joint and exhaust tube with foil.
3) Assemble supplies and samples under hood and/or utility cart. Label flasks.
4) Add five to six clean teflon chips into 500 mL round bottom flask.
5) Pour solvent into round bottom flask: 175 mL of acetone and 175 mL of hexane.
6) Transfer sample to soxhlet extractor:
   Vapor sample - XAD_2

Place glass wool plug at the siphon tube opening of the soxhlet extractor using glass or metal rod.

Carefully pour XAD_2 in soxhlet extractor. Rinse container with solvent (50% acetone/50% hexane) to remove all XAD_2; pour solvent rinse into soxhlet.

Assemble flask/soxhlet/condenser. Place on heating mantle.

Particle sample-Composite QF

Unwrap one QF at a time.

Trim off the number at the corner with clean scissors.

Use two pairs of blunt tweezers to fold one QF; place in soxhlet.

Rinse tweezers and scissors with CH_2Cl_2.

Repeat procedure for all QFs in composite sample.

Assemble flask/soxhlet/condenser. Place on heating mantle.

7) Spike extraction:

XAD_2 and QF Samples

Using a micropipette dispenser, spike each sample with 100 µL of the PCB surrogate standard. (One standard solution contains all three congeners.) 50 µL of dibutylchlorendate, 100 µL of terbutylazine, and 200 µL of d_{10} phenenthrene

Lab Blank

Using a micropipette dispenser, spike the extraction medium with 100µL of the PCB surrogate standard, 50 µL of dibutylchlorendate, 100 µL of terbuylazine, and 200 µL of d_{10} phenenthrene.

Combination matrix spike:

Spike sample medium with 1 mL of PCB recovery standard (683 ng of PCBs), 200 µL of Mixed Pesticide Recovery standard (20 ng of each), 200 µL of mixed PAH congeners (400 ng of each), 500 µL of atrazine (1000ng), 100 µL of PCB surrogate standard (14=20 ng, 65=5 ng, 166=5 ng), 50 µL of dibutyl chlorendate (25 ng), 100 µL of terbutylazine (560 ng), and 200 µL of d_{10} phenanthrene (400 ng). PCB recovery standard contains 683 ng of PCB in 1 mL (diluted from Michael D. Mullin 94 mix). These data are used for the recovery of individual PCB congeners, individual pesticides and PAHs.

8) Assemble flask/soxhlet/condenser unit. Place on heating mantle.
9) Turn on heating mantles: set Staco heating mantles to 45 or the multi-unit extraction heater to 5.
10) Turn on condenser water.
11) Cover soxhlet and flask with foil.
12) Extract for 18 to 24 hours.

Day 2

1) Turn heating mantle off. Let cool 15 to 30 minutes. Siphon off as much solvent from soxhlet extractor into flask as possible.
2) Detach the flask and insert stopper.
3) Turn off condenser water.
4) Store in cool dark place.

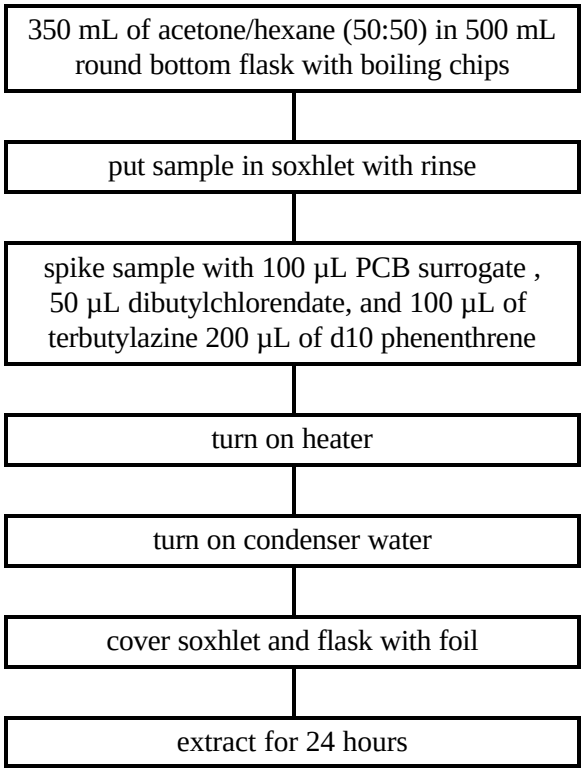
5.1.3 Comments

5.1.3.1 If XAD_2 gets into the flask, see Section 5.2.2. Removing XAD_2 from flask.

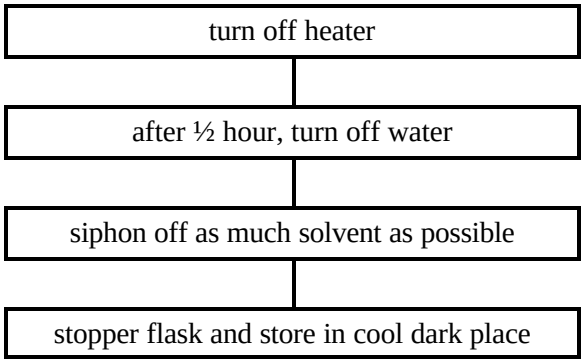
5.1.3.2 If condensation is a problem, wrap condensers with foil wrapped insulation or with kimwipes.

5.1.4 Flow charts for air sample extraction

5.1.4.1 Setting-up extraction



5.1.4.2 Taking down extraction



5.2 Rotary Evaporation

5.2.1 Supplies

5.2.1.1 After extraction/before column clean-up

5.2.1.1.1 *Glassware:*

Splash guard with 24/40 joint
100 or 200 mL beaker
Waste container for used boiling chips

5.2.1.1.2 *Non-glassware:*

Hexane
Clean large forceps
Cl^- and non-Cl^- waste bottles
CH_2Cl_2 in teflon bottle

5.2.1.1.3 *Equipment:*

Rotary evaporator
Aspirator pump
Chiller circulator

5.2.1.2 After column clean-up

5.2.1.2.1 *Glassware:*

Splash guards: one with 24/40 joint and one with 14/20 joint
25 mL beaker

5.2.1.2.2 *Non-glassware:*

Hexane
Cl^- and non-Cl^- waste bottles
CH_2Cl_2 in teflon bottle

5.2.1.2.3 *Equipment:*

Rotary evaporator
Aspirator pump
Chiller circulator

5.2.1.3 Removing XAD_2 from Flask

5.2.1.3.1 *Glassware:*

500 mL round bottom flask

5.2.1.3.2 *Non-glassware:*

Cork ring
Hexane

5.2.1.3.3 *Equipment:*

-none-

5.2.2 Procedures

5.2.2.1 Set-up

1) Fill chamber with DI water.
2) Turn on the chiller circulator.
3) Set bath temperature:

Solvent	Temperature (°C)
hexane	30-32
acetone	30-32
acetone/hexane	30-32
methanol	40
CH_2Cl_2	30

4) Rinse joint of steam duct with CH_2Cl_2.
5) Attach appropriate splash guard(s) to steam duct. Clamp each joint.
6) Turn on vacuum. Check vacuum of system.

5.2.2.2 Evaporation

1) Remove boiling chips with large forceps. If XAD_2 is in flask, see Section 5.2.2.4. Removing XAD_2 From Flask.
2) Attach flask to splash guard. Clamp joint.
3) Turn on motor of rotator to predetermined rotation speed (usually to the bottom of the indicator line, or about 50 rpm). Turn flask to start rotation. Evaporation should begin in approximately one minute; solvent should **not** boil.
4) Evaporate sample down to approximately 2 mL (in a 500 mL round bottom flask, area of liquid should be about the size of a quarter).
5) Open stopcock of rotary evaporator to release vacuum.
6) Detach the flask:

If exchanges are necessary, add specified amount of hexane from

Section 5.2.2.3. Solvent Exchanges, then return flask to splash guard and clamp.
If additional exchanges are not necessary, stopper flask. Store flask under cabinet.

7) Empty receiving flask into proper waste bottle as needed.
8) Rinse splash guard with CH_2Cl_2 before using with a different sample. Muffle splash guard at the end of a set of samples. Splash guards should be washed and muffled after every three or four sets of samples.

5.2.2.3 Solvent exchanges

	Fraction	Amount of Hexane to Add	No. of Exchanges	Total # of Rotary Evaporations	Final volume
after extraction	----	75 mL	2	3	2-5 mL
after column clean-up	hexane	----	0	1	1 mL
	50%	25 mL	1	2	1 mL

5.2.2.4 Removing XAD_2 from flask
1) Label another 500 mL flask with sample ID.
2) Decant sample from original flask into clean flask.
3) Rotary evaporate new flask using above procedures.
4) Add hexane for the exchanges to original flask with XAD_2; swirl hexane in flask to remove any remaining items of interest.
5) Decant hexane wash from original flask into new flask as needed to complete exchanges.

5.2.2.5 Clean-up
1) Turn off heater on rotary evaporator.
2) Turn off motor on rotary evaporator.
3) Turn off chiller.
4) Empty receiving flask into proper waste solvent bottle.
5) Cover steam duct with foil.

6.0 Rain Samples

6.1 Extraction

6.1.1 Supplies

6.1.1.1 *Glassware:*

Large soxhlet extractor (55/50 and 24/40 joints)
Condenser (55/50 joint)
500 mL round bottom flask
Glass stopper (24/40 joint)
Micro-dispenser (50 or 100 µL) and 1 mL pipette

200 mL (or larger) beaker

6.1.1.2 *Non-glassware:*

Boiling chips
Acetone
Hexane
CH_2Cl_2 in squirt bottle
Methanol in squirt bottle
Cl^- solvent waste bottle
Non-Cl^-solvent waste bottle
Cork ring for 500 mL flask
Glasswool
12" rod (glass or metal)
Large tweezers
Small tweezers
Foil
Spiking standards:

Standard	Concentration
PCB surrogate standard	Congener 14: 200 ng/ml
	Congener 65: 50 ng/mL
	Congener 166: 50 ng/mL
pesticide recovery standard	100 ng of each pesticide/mL
dibutylchlorendate	500 ng/mL
terbutylazine	5600 ng/mL
atrazine	2000 ng/mL
PAH recovery standard	2 µg of each PAH/mL
d_{10} phenanthrene	2 µg/mL
PCB recovery standard	683 ng of PCBs/mL

6.1.1.3 *Equipment:*

Heating mantle for 500 mL round bottom flask
Variable autotransformer or multi-unit extraction heater

6.1.2 Procedures

6.1.2.1 Extraction of Rain Samples from XAD_2 cartridges

One set of samples (usually one month's sample from all different site) is extracted on Day 1. A name is assigned to that set. An example of set name is Au94P-month of collection, year, and type of sample. In this case, P stands for precipitation sample. One set will include approximately six to eight samples, at

least one duplicate sample, one field blank, one lab blank, and one combination matrix spike.

Day 1

1) Remove spiking standards from freezer. Standards **must** be at ambient temperature before using. (Ambient temperature is achieved in about two hours.)
   - surrogate PCB standard
   - surrogate pesticide standard - dibutylchlorendate
   - surrogate atrazine standard - terbutylazine
   - surrogate PAH standarad - d_{10} phenanthrene
   - pesticide recovery standard
   - PAH recovery standard
   - PCB recovery standard
   - Atrazine recovery standard
2) Thoroughly rinse inside of condenser and outside of joint with solvent in squirt bottles: first with methanol, then with CH_2Cl_2. Cover joint and exhaust tube with foil.
3) Assemble supplies and samples under hood and/or utility cart. Label flasks.
4) Add five to six clean teflon chips into 500 mL round bottom flask.
5) Measure 175 mL acetone in a beaker.
6) Place glass wool plug at the siphon tube opening of the soxhlet extractor using glass or metal rod. Assemble soxhlet extractor and flask.
7) Put XAD_2 sample in soxhlet extractor. Rinse container with acetone from beaker; add this and remaining acetone from beaker to soxhlet.
8) Add 175 mL hexane to top of soxhlet.
9) Spike extraction:

   Samples

   Using micropipette dispenser, spike each sample with 100 µL of the PCB surrogate (One standard solution contains all three congeners), 50 µL of dibuylchlorendate, 100 µL of terbutylazine, and 200 µL of d_{10} phenanthrene.

   Lab Blank

   Using micropipette dispenser, spike approximately 8 g of clean XAD_2 with 100 µL of the PCB surrogate standard, 50 µL of dibuylchlorendate, 100 µL of terbutylazine, and 200 µL of d_{10} phenanthrene.

   Combination Matrix Spike or MS

   Spike sample medium with 1 ml of PCB recovery standard (683 ng of PCBs), 200 µL of Mixed Pesticide Recovery standard (20 ng of each), 200 µL of mixed PAH standard (400 ng of each), 500 µL of atrazine(1000 ng), 100 µL of PCB surrogate standard (14=20 ng, 65=5 ng, 166=5 ng), 50 µL of dibutyl chlorendate (25 ng), 100 µL of terbutylazine (560 ng), and 200 µL of d_{10} phenanthrene (400 ng). PCB recovery standard contains

683 ng of PCB in 1 mL (diluted from Michael D. Mullin 94 mix). These data are used for the recovery of individual PCB congeners, individual pesticides, each PAHs and atrazine.

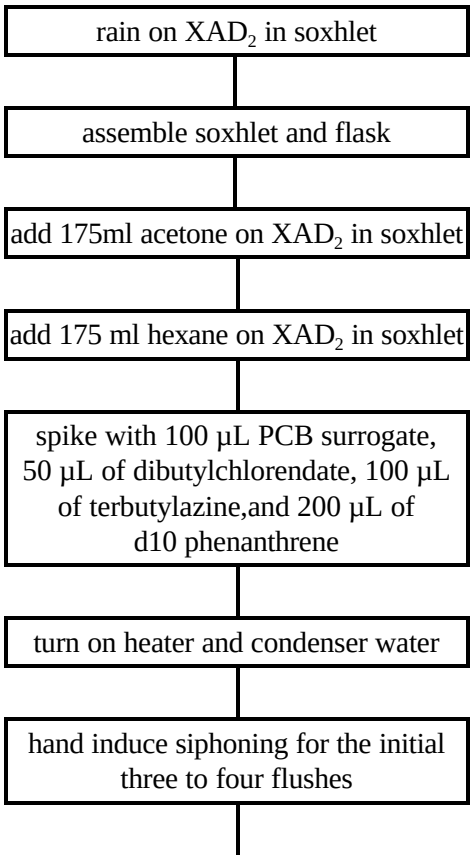
10) Assemble flask/soxhlet/condenser apparatus. Place on heating mantle.
11) Turn on heating mantles: set Staco heating mantle to 45 or the multi-unit extraction heater to 5.
12) Turn on condenser water.
13) Cover soxhlet and flask with foil.
14) Extract for 24 hours.

Note: The sample has water in it, thus it may not siphon on its own the first two or three times depending on the amount of water present. Induce siphoning until the level of solvent in the soxhlet and in the syphon tube are the same.

Day 2

1) Turn heating mantle off. Let cool 15 to 30 minutes. Siphon off as much solvent from soxhlet extractor into flask as possible.
2) Detach the flask and insert stopper.
3) Turn off condenser water.
4) Store in cool dark place.

6.1.3 Flow chart for the extraction of rain samples



extract for 24 hours

6.2 Rotary Evaporation

6.2.1 Supplies

6.2.1.1 After extraction/before column clean-up

6.2.1.1.1 *Glassware:*

Splash guard with 24/40 joint
100 mL or 200 mL beaker
Waste container for used boiling chips

6.2.1.1.2 *Non-glassware:*

Hexane
Clean large forceps
Cl^- and non-Cl^- waste bottles
CH_2Cl_2 in teflon bottle

6.2.1.1.3 *Equipment:*

Rotary evaporator
Aspirator pump
Chiller circulator

6.2.1.2 After column clean-up

6.2.1.2.1 *Glassware:*

Splash guards: one with 24/40 joint and one with 14/20 joint.
25 mL beaker

6.2.1.2.2 *Non-glassware:*

Hexane
Cl^- and non-Cl^- waste bottles
CH_2Cl_2 in teflon bottle

6.2.1.2.3 *Equipment:*

Rotary evaporator
Chiller circulator

6.2.1.3 Back Extraction (in addition to the items listed in Section 6.2.1, Supplies. After extraction/before column clean-up)

6.2.1.3.1 *Glassware:*

125 mL separatory funnel
Pasteur pipettes
10 mL graduated pipette

6.2.1.3.2 *Non-glassware:*

Rubber pipette bulb

6.2.1.3.3 *Equipment:*

Three-prong clamp with support

6.2.2 Procedures

6.2.2.1 XAD_2 Cartridges

6.2.2.1.1 Set-up

1) Fill chamber with DI water.
2) Turn on the chiller circulator.
3) Set bath temperature:

Solvent	Temperature (°C)
hexane	30-32
methanol	40
acetone/hexane	30-32
CH_2Cl_2	30

4) Rinse joint of steam duct with CH_2Cl_2.
5) Attach appropriate splash guard(s) to steam duct. Clamp each joint.
6) Turn on vacuum. Check vacuum of system.

6.2.2.1.2 Evaporation

1) Remove boiling chips with large forceps. If XAD_2 is in flask, see Section 6.2.2.1.3. Removing XAD_2 from flask.
2) Attach flask to splash guard. Clamp joint.
3) Turn on motor of rotator to predetermined rotation speed (usually to the bottom of the indicator line, or about 50 rpm). Turn flask to start rotation. Evaporation should begin in approximately one minute; solvent should **not** boil.
4) Evaporate sample until the evaporation slows down. **Note:** If rate of evaporation slows down, **DO NOT** continue. There is water in the sample.

6.2.2.1.3 Removing XAD_2 from the flask

1) Label another 500 mL flask with the samples ID
2) Decant sample from original flask into the clean flask; wash with 10 mL hexane twice.
3) Rotary evaporate the new flask until evaporation begins to slow down.

6.2.2.1.4 Back extraction and solvent exchanges

1) Add 75 mL hexane to sample flask. Rotavap again to 100 mL. Transfer the content to separatory funnel. Add 1 gm of sodium sulfate. Shake vigorously. Wait for 20 minutes.
2) First Extract:
   Transfer the original hexane layer to the flask. Add 25 mL hexane to the water in separatory funnel. Then add approximately 1 g of Na_2SO_4. Shake vigorously; let stand <u>at least</u> 20 minutes. While waiting for first extract to separate, rotary evaporate the original sample to approximately 5 mL.
   After 20 minutes or so, pipette the hexane out and add it to the original sample flask.
3) Second Extract:
   Add 25 mL hexane to the water layer in the separatory funnel. Shake vigorously; let stand <u>at least</u> 20 minutes.
   Pipette out the hexane layer from the separatory funnel; add it to the original flask.
4) Third Extract:
   Add 25 mL hexane to the water layer in the separatory funnel. Shake vigorously; let stand <u>at least</u> 20 minutes.
   Pipette out the hexane layer from the separatory funnel; add it to the original flask.
5) Rotary evaporate the combined extract to 2 mL.

Note: *****More water may separate out after the addition of the first and second extract. Pipette the water out and add it to the separatory funnel.

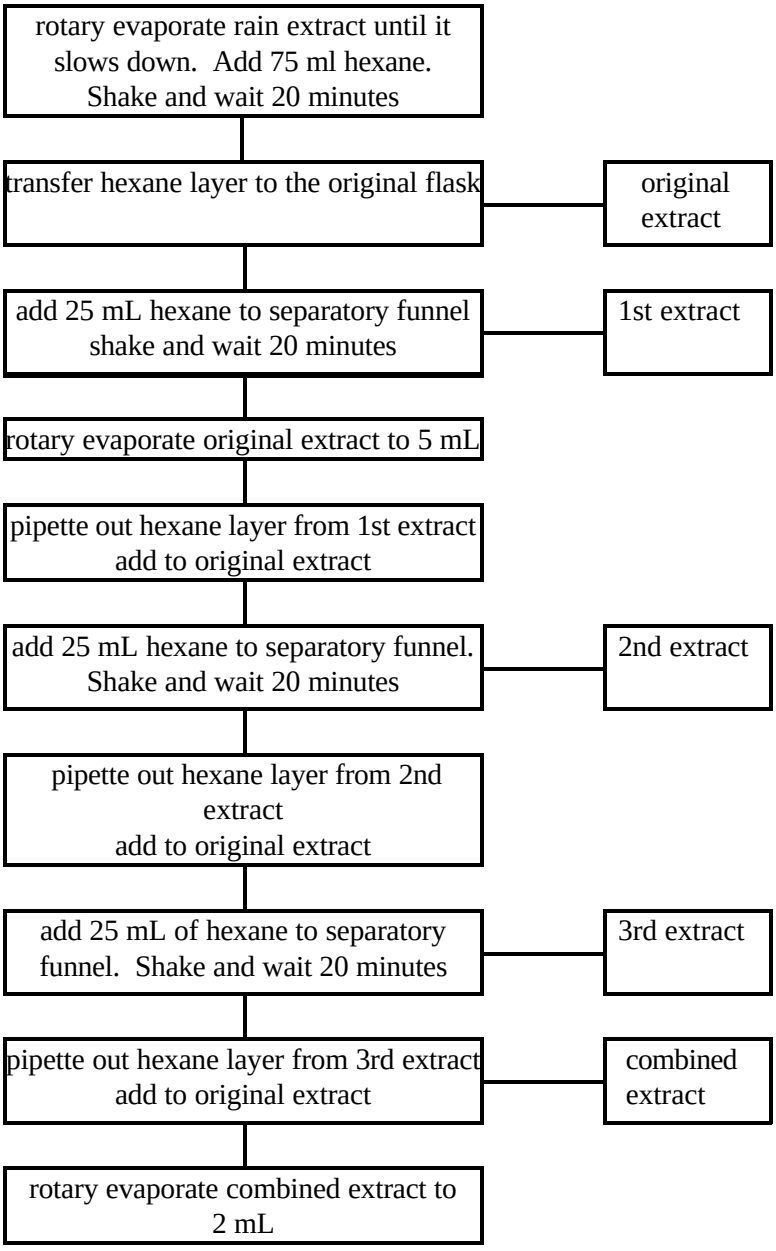
*****It is possible trace amounts of water may be in the final sample - ignore it! The $NaSO_4$ on the top of the silica column will take care of it.

*****If an emultion forms in the separatory funnel, add extra Na_2SO_4 to the funnel. This will facilitate the separation of the water.

6.2.2.1.5 Clean-up

1) Turn off heater on rotary evaporator.
2) Turn off motor on rotary evaporator.
3) Turn off chiller.
4) Empty receiving flask into proper waste solvent bottle.
5) Cover steam duct with foil.

6.2.2.1.6 Flow Chart of Rotary Evaporation and Back Extraction



6.2.2.2 Rotary evaporation after column chromatography

1) Attach flask to splash guard. Clamp joint.
2) Turn on motor of rotator to pre-determined rotation speed (usually to the bottom of the indicator line, or about 50 rpm). Turn flask to start rotation. Evaporation should begin in approximately one minute; solvent should **not** boil.
3) Evaporate sample down to approximately 2 mL.
4) Open stopcock of rotary evaporator to release vacuum.
5) Detach the flask:
   If exchanges are necessary, add specified amount of hexane as listed in Section 5.2.2.3, Solvent Exchanges, then return flask to splash guard and clamp.
   If additional exchanges are not necessary, stopper flask and store it under the cabinet.
6) Empty receiving flask into proper waste bottle as needed.
7) Rinse splash guard with CH_2Cl_2 before using with a different sample. Muffle splashguard a the end of a set of samples. Splash guards should be washed and muffled after every three or four sets of samples.

6.2.2.3 Solvent Exchanges

	Fraction	Amount of Hexane to Add	No. of Exchanges	Total # of Rotary Evaporations	Final Volume
after column chromatography	hexane	----	0	1	1 mL
	50%	25 mL	1	2	1 mL
	meth.	-----	0	0	1 mL

7.0 Silica Column Chromatography

7.1 Supplies

7.1.1 Activation/Deactivation

7.1.1.1 *Glassware:*

100 mL or 250 mL beaker
Powder funnel
250 mL or 500 mL round bottom flask
Stopper to fit round bottom flask
1 mL graduated pipette
25 mL beaker

7.1.1.2 *Non-glassware:*

*****Silica
Pipette bulb
Cork ring to fit round bottom flask

7.1.1.3 *Equipment:*

Muffle furnace
Desiccator
Calculator
Balance
Particle mask

7.1.2 Column clean-up (for a three-fraction column clean-up of one sample)

7.1.2.1 *Glassware:*

Column
Three 100 mL pear shaped flasks with 14/20 joints
Three glass stoppers with 14/20 joints
Pasteur pipettes (9½ inch and/or 5¼ inch): minimum of one for each sample and six additional pipettes for each set of samples fractionated
Graduated cylinders: 50 mL and 10 mL
Funnel
100 mL beaker
250 mL beaker OR waste jar (need not be clean)
Three 250 mL beakers

7.1.2.2 *Non-glassware:*

Rubber pipette bulbs
Hexane
50% hexane/50% CH_2Cl_2
CH_2Cl_2
Methanol
Two cork rings for 100 mL pear shaped flasks (size #1)
Rubber hammer
Stainless steel spatula
20" rod
Teflon stopcock
Glass wool
4% deactivated silica
$NaSO_4$

7.1.2.3 *Equipment*

Ultrasonicator

7.1.3 Supply chart for <u>each</u> sample

Item	Air Particle (QF)	Air Vapor (XAD_2)	Rain (XAD_2)
amount of silica to activate/deactivate	4-6 g	4-6 g	4-6 g
column size	3.5"	3.5"	3.5"
amount of $NaSO_4$	0.5	0.5"	1.5"
elution volume (1st and 2nd fraction)	25 mL	25 mL	30 mL
switching volume	4 mL	4 mL	5 mL
elution volume (3 rd fraction)	30 mL	30 mL	35 mL

7.2 Procedures

7.2.1 General procedures

7.2.1.1 Activation/Deactivation of silica

Day 1

1) Place approximate amount of silica needed in a beaker. Cover beaker with foil.
2) Place beaker in 100°C oven, turn thermostat to 300°C; keep in oven overnight.
DO <u>NOT</u> PUT SILICA INTO 300°C OVEN!

Day 2

1) Turn oven temperature down to 100°C;
DO NOT REMOVE SILICA FROM OVEN.
2) When oven has cooled to 100°C, remove beaker from oven; let cool on counter top until warm (approximately 5 to 10 minutes); place in desiccator.
3) When silica has reached ambient temperature (approximately two hours), deactivate it:

Working quickly, weigh out desired amount of silica in the round bottom flask. Stopper flask **<u>immediately</u>** after pouring silica.Add 4% weight/volume of DI water to silica, using the following equation:

$$\frac{\%\ deactivation}{100\ -\ \%\ deactivation} = \frac{mL\ DI\ water}{weight\ of\ silica\ (gm)}$$

For precipitation samples use 3% deactivation.

SHAKE WELL. Shake flask until all clumps are broken-up. Store in desiccator overnight for equilibration. Use deactivated silica in desiccator within three days. Any unused silica may be reused after re-activating and re-deactivating).

7.2.1.2 Preparation and packing of column(s)

1) Assemble stopcock(s) on column(s).
2) Stuff glass wool plug (approximately 1 cm) into lower end of the each column with 20" rod.
3) Measure and mark appropriate distance from top of glass wool plug.
4) Clamp column(s) securely onto frame in ventilation hood. Place empty glass container under each column (100 mL minimum size; it need not be clean).
5) Close stopcock(s); fill column(s) half full with hexane.
6) Make a slurry of hexane and deactivated silica. Pour slurry into each column. **DO NOT ALLOW SILICA TO DRY OUT**: rinse column and beaker with hexane via Pasteur pipette. (Use of a funnel may facilitate process.) Open stopcock(s). Tap column(s) with rubber hammer to pack silica. Add silica/hexane as needed until desired length is loaded.
7) Cap column(s) with ½" Na_2SO_4 for XAD_2 and QF samples, 0.5" Na_2SO_4 for rain samples.
8) Wash column(s) with 25 mL hexane for conditioning.
9) When hexane level reaches 1 cm above top of Na_2SO_4, close stopcock(s) to prevent further dripping. **NEVER LET COLUMN RUN DRY.**
10) If column(s) is/are not going to be used immediately, stopper column(s) and cover tip(s) of column(s) with foil.

7.2.1.3 Set-up

1) Label one 100 mL pear-shaped flask for each fraction which is to be collected.
2) On a cart, assemble pear shaped flasks and remaining supplies listed in Section 7.1, Supplies.
3) Place sample flask in front of column.
4) Place a 50 mL or 100 mL beaker in front of sample flask.
5) Add hexane to 50 mL or 100 mL beaker; cover with foil. (For volume of hexane, see chart in Section 7.1.3, Supply Chart.)

7.2.1.4 Column chromatography

7.2.1.4.1 First fraction

1) Ultrasonicate sample flask before loading the sample onto the column to detach the particles which are sticking to the walls of the flask.

2) Remove stopper from sample flask. Assemble pipette and rubber bulb; place pipette in sample flask.
3) Place Fraction #1 (hexane) pear shaped flask under the column.
4) Open stopcock and let column drip until hexane level is at the top of the Na_2SO_4.
5) Load sample into column with Pasteur pipette.
6) Set drip rate to approximately one drip per second. Add approximately 5 mL hexane to sample flask from the beaker. Swirl solvent in flask..
7) When sample has drained down to the top of the Na_2SO_4, add the hexane from the sample flask to the column. Add an additional 5 mL hexane to the sample flask from the beaker. Swirl solvent in flask.
8) When solvent has drained down to the top of the Na_2SO_4, add the second 5 mL hexane to the column. Add the remaining hexane from the beaker to the sample flask. Swirl solvent in sample flask.
9) When solvent has drained down to the top of the Na_2SO_4, add the remaining hexane from the sample flask. (If reservoir on top of the column cannot hold entire amount, add as much as possible, then refill as space becomes available.)
Note: Stagger the timing of the column loadings such that the changing of the flasks are not concurrent.

7.2.1.4.2 Second fraction

1) While the hexane is dripping (from the first fraction), measure the hexane/CH_2Cl_2 and put it into the appropriate containers.
2) When the hexane drips down to the top of the Na_2SO_4, add the switching volume hexane/CH_2Cl_2 from the sample flask to the column.
3) Transfer the hexane/CH_2Cl_2 from the beaker to the sample flask. Swirl the solvent in the flask.
4) Place the appropriate pear shaped flask (labeled '50%' fraction) next to the flask under the column.
5) When the hexane/CH_2Cl_2 level in the column is to the top of the $NaSO_4$, quickly switch flasks and pour as much of the remaining hexane/CH_2Cl_2 into the column as possible. Add hexane/CH_2Cl_2 to the column as space permits.
6) Continue to monitor the rate of drip (approximately one drip per minute).
7) Place the pear shaped flask from the first fraction on the supply cart. Stopper the flask.
8) Once the column has stopped dripping, remove flask from second fraction, stopper it, and put it on the supply cart.

9) collect another fraction with 30 ml of methanol in case of air samples and 35 mL of methanol in case of rain samples.

7.2.1.4.3 Clean-up

1) Remove stopcock from column.
2) Turn column upside down and secure it with clamps. Place container under column to catch $NaSO_4$ and silica.
3) After column has dried out, use vacuum (air or water) to remove glass wool plug.
4) Pour silica and $NaSO_4$ into used glove or foil before discarding into trash can.

7.2.2 Specific procedures by sample type

7.2.2.1 XAD_2 (vapor) and GFF

Follow General Procedures.

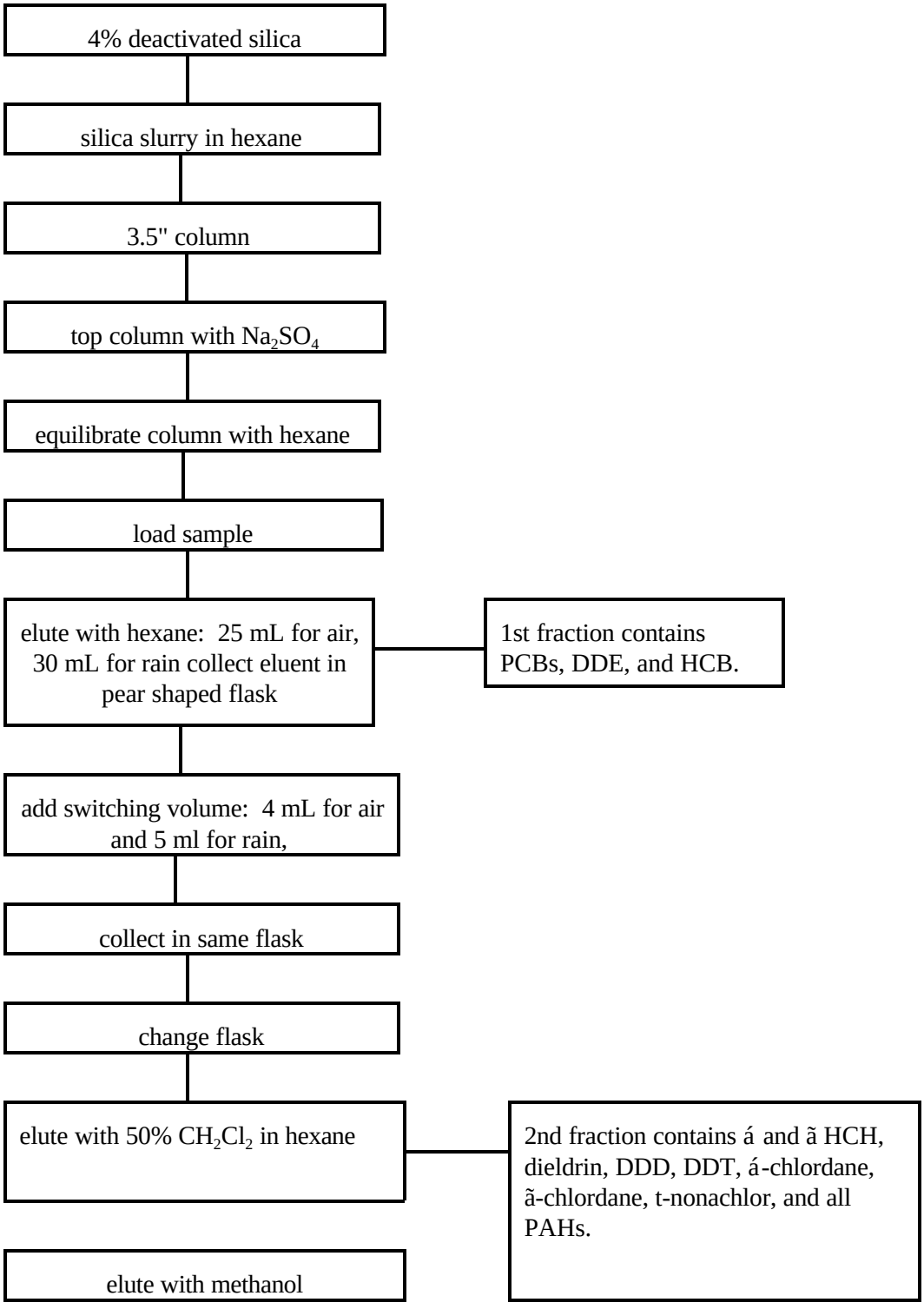
7.2.2.2 Rain samples

Procedure is essentially the same as XAD_2 (vapor and particles) and QF with the following exceptions:

1) $NaSO_4$ should be activated no more than 2 to 3 days before using; cap should be 1.5".
2) Elution volumes:

Solvent	Solvent needed (mL) for rain samples
hexane	30
50% CH_2Cl_2 in hexane	30
switching volume	5
methanol	35

7.2.3 Summary flow-chart



8.0 Transfer of Solution

8.1 Supplies for Each Sample

8.1.1 *Glassware:*

Pasteur pipettes (9½ inch and/or 5¼ inch): minimum of one for each fraction and one additional pipette for hexane
4 mL amber glass vial for each fraction
50 mL beaker

8.1.2 *Non-glassware:*

Vial file for 4 mL vials
Rubber pipette bulbs
Hexane (minimum 2 mL for each fraction)

8.1.3 *Equipment:*

-none-

8.2 Procedures

8.2.1 Preparation

1) Label each amber vial with sample ID and fraction ID.
2) Put hexane in 50 mL beaker (approximately 2 mL per sample).
3) Concentrate all fractions by rotary evaporation to 1 mL. 50% CH_2CL_2 fraction needs to be solvent exchanged to hexane (see Section 5.2.3).

8.2.2 Transferring sample

1) Using a Pasteur pipette, transfer entire sample from flask to amber vial.
2) Add approximately 1 mL hexane to flask and swish solvent around to clean out flask; transfer to amber vial.
3) Repeat.
   Note: Do not add so much solvent as to fill the vial. If it is too full, there is a chance of splashing at the time of N_2 blowdown.
4) Close amber vial tightly, place in vial file, and store in freezer. Label the vial file with sample set name, type of samples, and site of collection.

9.0 N_2 Blowdown

9.1 Supplies

9.1.1 *Glassware:*

-none-

9.1.2 *Non-glassware:*

CH_2Cl_2
Sample in amber vial

9.1.3 *Equipment:*

N_2 blowdown unit

9.2 Procedures

9.2.1 Set-up

1) Remove all nozzle plugs from unit.
2) Turn on N_2 at tank and trap (do NOT touch primary or secondary controls on regulator). Let N_2 flush out for approximately five minutes.
3) Turn heater on LOW.
4) Attach clean needle to each nozzle to be used.

9.2.2 Blowdown

1) Place amber vials in slot; adjust N_2 flow such that there are gentle (barely detectable) ripples in the vials.
2) Evaporate down to the approximate predetermined volume. (See following chart.)

Type of sample		Approximate volume after N_2 blowdown (mL)		
		hexane fraction	50% fraction	methanol fraction
rain	winter	0.4	0.4	0.4
	summer	0.4	0.4	0.4
QF	winter	0.4	0.4	0.4
	summer	0.4	0.8	0.4
XAD_2	winter	0.4-0.8	1.0-2.0	0.4
	summer	0.8-1.0	1.5-2.0	0.4

9.2.3 Closing-up unit

1) Turn off N_2 at trap and near the regulator.
2) Replace the nozzle caps.
3) Rinse nozzle extension tubes with CH_2Cl_2. After three to four uses or after a highly contaminated batch of samples, ultrasonicate nozzle extension tubes.

10.0 Spiking Samples with ISTD

10.1 Supplies

10.1.1 *Glassware:*

-none-

10.1.2 *Non-glassware:*

Samples in 4 mL amber glass vials
Internal standards (see next page)
Hexane
CH_2Cl_2
Cl^- and non-Cl^- waste containers

10.1.3 *Equipment:*

25, 50, and 100 µL microdispensers

10.2 Procedures

1) Remove ISTDs from freezer; equilibrate to ambient temperature (approximately two hours).

Fraction	Compound	Type of sample	Internal Standard	Spike Volume (µL)	Final Mass in Sample (ng)	Color of Dot on Label
hexane	PCBs and pesticides	vapor, particle, and rain	PCB 30	100	8	red
			PCB 204		6	
50%	PAHs	vapor, particle, and rain	D_{10} anthracene,	50	200	black
			D_{12} benzo(a)anthracene		200	
			triphenylmethane		134.6	
			d_{12} perylene		180	
50%	Pesticide	vapor particle rain	PCB 65	100	20	blue
			PCB 155		20	
methanol	atrazine	vapor particle rain	d_{10} anthracene	50	200	green

2) Clean micropipette

Remove glass tube used to cover plunger.
Rinse plunger with CH_2Cl_2. Wave pipette to evaporate solvent.
Without touching glass tubes, insert plunger into new glass capillary; tighten tube in place.
Rinse the capillary with dichloromethane twice and air dry. Draw spiking standard. Make sure that there is no air bubble.

3) Spike sample vial (see above chart for surrogate and amount).
4) Mark each amber vial label with a appropriate color of dot (use a water-proof marker).
5) Replace glass tube used to cover plunger of micropipette. Store micropipette.

11.0 Making Microvials for GC Analysis

11.1 Supplies

11.1.1 *Glassware:*

Conical microvials
Pasteur pipettes

11.1.2 *Non-glassware:*

Vial racks
Septa (vial caps)

11.1.3 *Equipment:*

Crimper

11.2 Procedures

1) Label conical microvials with sample Ids. In addition, label extra microvial for hexane and the appropriate calibration standard for every set of samples.
2) Using a Pasteur pipette, remove approximately 200 µL of each sample and put in labeled conical microvial. (The level of liquid will be at the shoulder of the microvial.) Also place 200 µL of hexane and 200 µL of the appropriate standard into the labeled microvials. (See following chart for the appropriate standard.)

Fraction	Target Compounds	Calibration Standard
hexane	PCBs	Mullin 94: 683 ng/mL
50%	pesticides	mixed pesticide standard: 20 ng/mL ea
50%	PAH	mixed PAH standard: 200 ng/mL ea (approx)
methanol	atrazine	1000 ng/mL

Note: Make one vial with performance standard for each set of analyte.

3) Crimp septa onto microvial.

4) Load microvials into autosampler or store in freezer.

12.0 Standards

12.1 PCB Standards

12.1.1 Mullin's 94 mix: 170.8 µg/mL: Mixture of 1232, 1248, and 1262 in 25:18:18.

12.1.2. Surrogate standards

12.1.2.1 Congener 14: Primary stock: 100 µg/mL in isooctane: Accustandard.

	Primary Stock	Dilution	Final Concentration
Secondary stock	1 mL (100 µg)	to 100 mL hexane	1000 ng/mL

12.1.2.2 Congener 65: Primary stock: 100 µg/mL in isooctane: Accustandard

	Primary Stock	Dilution	Final Concentration
Secondary stock	1 mL (100 µg)	to 100 mL hexane	1000 ng/mL

12.1.2.3 Congener 166: Primary stock: 100 µg/mL in isooctane: Accustandard

	Primary Stock	Dilution	Final Concentration
Secondary stock	1 mL (100 µg)	to 100 mL hexane	1000 ng/mL

12.1.2.4 PCB mix surrogate recovery standard: To be used for spiking each sample.

Congener	Stock Concentration	Mix	Final Concentration
14	1000 ng/mL	10 mL	200 ng/mL
65	1000 ng/mL	2.5 mL	50 ng/mL
166	1000 ng/mL	2.5 mL	50 ng/mL

Volume was made up to 50 mL with hexane.

12.1.3 Internal Standards (ISTD)

12.1.3.1 Congener 30: Primary stock: 100 µg/mL in isooctane: Accustandard

	Primary Stock	Dilution	Final Concentration
Secondary stock	0.5 mL (50 µg)	50 mL	1000 ng/mL

12.1.3.2 Congener 204: Primary stock: 100 µg/mL in isooctane: Accustandard

	Primary Stock	Dilution	Final Concentration
Secondary stock	0.5 mL (50 µg)	50 mL	1000 ng/mL

12.1.3.3 PCB spiking standard:

Congener	Stock Concentration	Mix	Final Concentration
30	1000 ng/mL	8 mL	80 ng/mL
204	1000 ng/mL	6 mL	60 ng/mL
Volume was made up to 100 mL with hexane.			

12.1.4 PCB calibration standard for PCBs

Congener	Stock Concentration	Mix	Final Concentration
Mullin 94	170.8 µg/mL	400 µL	683.2 ng/mL
14	1000 ng/mL	2 mL	20 ng/mL
65	1000 ng/mL	0.5	5 ng/mL
166	1000 ng/mL	0.5	5 ng/mL
30	1000 ng/mL	0.8	8 ng/mL
204	1000 ng/mL	0.6	6 ng/mL
DDE	2000 ng/mL	1 mL	20 ng/mL
HCB	2000ng/mL	1 mL	20 ng/mL
Volume made up to 100 mL with hexane.			

12.1.5 PCB recovery standard for PCBs: used for matrix spike.

Congener	Stock Concentration	Mix	Final Concentration
Mullin 94	170.8 µg/mL	400 µL	683.2 ng/mL

12.1.6 PCB performance standard: used for instrument calibration check.

Congener	Stock Concentration	Mix	Final Concentration
Mullin 94	170.8 µg/mL	300 µL	512.4 ng/mL
14	1000 ng/mL	1 mL	10 ng/mL
65	1000 ng/mL	1 mL	10 ng/mL
166	1000 ng/mL	1 mL	10 ng/mL
30	1000 ng/mL	0.8	8 ng/mL
204	1000 ng/mL	0.6	6 ng/mL
DDE	2000 ng/mL	0.5 mL	10 ng/mL
HCB	2000ng/mL	0.5 mL	10 ng/mL
Volume made up to 100 mL with hexane			

12.2 Pesticide Standards

12.2.1 Stock solutions

12.2.1.1 Primary stock

12.2.1.2 Stock

Pesticide	Ultra Sc. ampule concentration	Dilution	Stock Concentration
dieldrin	100 µg/mL in MeOH	1 mL 50 mL hexane	2 µg/mL
á-HCH	100 µg/mL in MeOH	1 mL 50 mL hexane	2 µg/mL
ã-HCH	100 µg/mL in MeOH	1 mL 50 mL hexane	2 µg/mL
HCB	100 µg/mL in methylene chloride	1 mL 50 mL hexane	2 µg/mL

Pesticide	Ultra Sc. ampule concentration	Dilution	Stock Concentration
4-4'DDT	100 µg/mL inMeOH	1 mL 50 mL hexane	2 µg/mL
4-4'DDD	100 µg/mL in MeOH	1 mL 50 mL hexane	2 µg/mL
4-4'DDE	100µg/mL in MeOH	1 mL 50 mL hexane	2 µg/mL
á-chlordane	100 µg/mL in MeOH	1 mL 100 mL hexane	1 µg/mL
ã-chlordane	100 µg/mL in MeOH	1 mL --- 100 mL in hexane	1 µg/mL
t-nonachlor	100 µg/mL in MeOH	1 mL ---- 100 mL in hexane	1 µg/mL
atrazine	100 µg/mL MeOH	1 mL 50 mL hexane	2 µg/mL

12.2.2 Pesticide spiking standard: Cong 65, 155

Compound	Stock Concentration	Mix	Final Concentration
Congener 65	1000 ng/ mL	10 mL	200 ng/mL
Congener 155	1000 ng/ mL	10 mL	200 ng/mL
Volume made up to 50 mL with hexane			

12.2.3 Pesticide surrogate standard

Dibutylchlorendate: 100 µg/ mL in methanol
Stock: 1 mL of above diluted to 100 mL in hexane = 1000 ng/ mL
Spiking standard: 25 mL of stock solution diluted to 50 mL with hexane = 500 ng/ mL

Terbutylazine: 2.8 mg was weighed and diluted to 100 mL with MeOH
Stock: 28000 ng/ mL
Spiking standard: 10 mL of stock was diluted to 50 mL with CH_2CL_2 = 5600 ng/mL

12.2.4 Mixed pesticide calibration standard: MPS 65, 155

This is used for analysis of pesticides in 50% CH_2Cl_2 fraction.

Compound	Stock Concentration	Mix	Final Concentration
á-HCH	2000 ng/mL	1 mL	20 ng/mL
ã-HCH	2000 ng/mL	1 mL	20 ng/mL
dieldrin	2000 ng/mL	1 mL	20 ng/mL
DDT	2000 ng/mL	1 mL	20 ng/mL
DDD	2000 ng/mL	1 mL	20 ng/mL
á-chlordane	1000 ng/mL	2 mL	20 ng/mL
ã-chlordane	1000 ng/mL	2 mL	20 ng/mL
t-nonachlor	1000 ng/mL	1 mL	20 ng/mL
Cong. 155	1000 ng/mL	2 mL	20 ng/mL
Cong. 65	1000 ng/mL	2 mL	20 ng/mL
Volume made up to 100 mL with hexane			

12.2.5 Mixed pesticide performance standard

Compound	Stock Concentration	Mix	Final Concentration
Pest Recovery standard B5 from ISWS	100 ng of each / mL	5 mL	10 ng of each/ mL
Dibutyl chlorendate	1000 ng/ mL	0.5 mL	10 ng/ mL
cong 65	1000 ng/ mL	0.5 mL	10 ng/ mL
cong 155	1000 ng/ mL	0.5 mL	10 ng/ mL

Volume was made up to 50 mL with hexane.

12.2.6 Pesticide recovery standard

Compound	Stock Concentration	Mix	Final Concentration
HCB	2000 ng/mL	2.5 mL	100 ng/mL
á-HCH	2000 ng/mL	2.5 mL	100 ng/mL
ã-HCH	2000 ng/mL	2.5 mL	100 ng/mL
dieldrin	2000 ng/mL	2.5 mL	100 ng/mL
4-4' DDE	2000 ng/mL	2.5 mL	100 ng/mL
4-4' DDD	2000 ng/mL	2.5 mL	100 ng/mL
4-4' DDT	2000 ng/mL	2.5 mL	100 ng/mL
á-chlordane	1000 ng/mL	5 mL	100 ng/mL
ã-chlordane	1000 ng/mL	5 mL	100 ng/mL
t-nonachlor	1000 ng/mL	25 mL	100 ng/mL
Volume made up to 50 mL with hexane			

12.3 PAH Standard

12.3.1 PAH mixed GC/MS calibration standard
solvent = hexane

PAH	Stock Conc. (µg/mL)	mL stock	Final Volume (mL)	Final Conc. (µg/mL)
acenapthene	1.97	10	100	0.20
acenapthylene	1.97	10	100	0.20
anthracene	1.97	10	100	0.20
benzo(a)anthracene	1.97	10	100	0.20
benzo(a)pyrene	1.97	10	100	0.20
benzo(b)fluoranthene	1.97	10	100	0.20
benzo(e)pyrene	1.91	10	100	0.19
benzo(g,h,i)perylene	1.97	10	100	0.20
benzo(k)fluoranthrene	1.97	10	100	0.20
chrysene	1.97	10	100	0.20
coronene	1.93	10	100	0.19

PAH	Stock Conc. (µg/mL)	mL stock	Final Volume (mL)	Final Conc. (µg/mL)
d_{10}anthracene-ISTD	4.00	4.2	100	0.17
d_{10} perylene	3.6	4.2	100	0.15
d_{12}benzo(a)anthracene-ISTD	4.00	4.2	100	0.17
dibenzo(a,h)anthracene	1.97	10	100	0.20
fluoranthene	1.97	10	100	0.20
fluorene	1.97	10	100	0.20
indeno(1,2,3,cd)pyrene	1.97	10	100	0.20
napthalene	1.97	10	100	0.20
phenanthrene	1.97	10	100	0.20
pyrene	1.97	10	100	0.20
retene	1.98	10	100	0.20
triphenylmethane-ISTD	2.69	4.2	100	0.11

12.3.2 PAH matrix spike recovery standard, Batch 2A

Analyte	Stock Conc. (µg/mL)	Stock Amt. (mL)	Final Conc. (µg/mL)
Acenapthene	100	1.97	1.97
Acenapthylene	100	1.97	1.97
Anthracene	100	1.97	1.97
Benzo(a)anthracene	100	1.97	1.97
Benzo(b)fluoranthene	100	1.97	1.97
Benzo(k)fluroanthene	100	1.97	1.97
Benzo(a)pyrene	100	1.97	1.97
Benzo(e)pyrene	96.5	1.98	1.91
Benzo(g,h,i)perylene	100	1.97	1.97
Chrysene	100	1.97	1.97
Coronene	98.2	1.97	1.93
Dibenz(a,h)anthracene	100	1.97	1.97

Analyte	Stock Conc. (µg/mL)	Stock Amt. (mL)	Final Conc. (µg/mL)
Fluoranthene	100	1.97	1.97
Fluorene	100	1.97	1.97
Indeno(1,2,3,cd)pyrene	50	1.97	1.97
Naphthalene	95.24	1.97	1.97
Phenanthrene	100	1.97	1.97
Pyrene	100	1.97	2.0
Retene	158.95	1.98	1.98

Volume was made up to 100 mL with hexane.

12.3.3 PAH internal standard

Compound	Stock (µg/mL)	Mix (mL)	Final Concentration (µg/mL)
d_{10}anthracene	1000	0.2	4
d_{12}benzo(a)anthracene	1000	0.2	4
d_{12} perylene	2000	0.09	3.60
triphenylmethane	136	0.99	2.69
Volume was made up to 50 mL with hexane			

12.3.4 PAH surrogate standard

d10 Phenanthrene: 2.13 ug/ mL of hexane

13.0 Safety

13.1 Emergency Numbers

Name	***Telephone numbers***
IU Fire Department	911
Ronald A. Hites	812-855-0193 (O) 812-334-1323 (H)
Jeffery White	812-855-1466 (O) 812-336-1462 (H)

13.2 Chemists Numbers

Name	*Telephone Numbers*
Ilora Basu	812-855-5040 (O) 812-855-2926 (O) 812-334-2184 (H)
Barbara Hillery	812-855-1005 (O) 812-334-4151 (H)
James M. O'Dell	812-855-5040 (O) 812-824-7962 (H)
Tom Stanko	812-855-2926 (O) 812-336-8546 (H)
Mary Tankard	812-855-5035 (O) 812-824-1863 (H)
Mike Wassouf	812-855-2926 (O) 812-330-1517 (H)
Charles Alan Long	812-855-2926 (O) 812-333-9535 (H)

13.3 Working in the Laboratory

Chemists working in the laboratory should follow certain safety rules :

1) Individual is required to wear a lab coat whenever working in the lab.
2) Eye protection with splash resistant safety glasses or safety goggles is required. Contact lens is forbidden.
3) Protective gloves should be used while handling samples or standards. Special solvent resistant gloves should be used while handling large amount of solvents.
4) All solvent work should be done inside fume hood.
5) Open shoes are not allowed in the laboratory.
6) Particle mask is required when using dry silica.
7) Generally nobody should work alone in the laboratory. If work must be performed after hours or in the weekend inform supervisor or other lab mates so that your presence is known and will be accounted for in case of an emergency.
8) Chemicals and solvents are stored in separate storage area. One week's supply is kept in the lab. Solvents are stored in special solvent cabinet. Acids must be separated from bases. A rubber bucket needs to be used to carry any chemical.
9) Gas cylinders should be well secured at all times. Flammable gases are stored in separate cage.

10) Wash your hands well after work. Protective hand cream "Soft guard" is supplied.
11) No food or drink is allowed in the laboratory.

12) In case of minor spillage, get spillage kit to clean the area. A major spill requires the University Health and Safety Division to be contacted and the working area evacuated.
13) MSDS are filed in a three ring binder.
14) All chemicals and standard should be labeled properly with scientific name, date, and initials of person to contact.
15) Empty chemical bottles should be flushed out with water, or, in case of liquid, allowed to evaporate under a hood before discarding.

13.4 Safety Equipment

13.4.1 Fume hood

IADN sample preparation requires frequent use of solvent. Therefore, all extraction, column chromatography, standard preparation, sample transfer, Nitrogen blow down and preparation of microvials should be done in the hood. It is real important to check hood from time to time to ensure that it is working properly. A flow of 80-120 linear feet per second must cross the hood.

13.4.2 Safety showers

Emergency showers are located in strategic areas of the laboratory to provide to provide immediate emergency protection against fire or chemical injury. It is operated by pulling the hanging ring down. It delivers 30 gallons of water per minute.

13.4.3 Eye Wash

Emergency eye wash is located in the laboratory. It is operated by pushing the lever backward.

13.5 Waste Disposal

13.5.1 Solvents

1) Label 2 containers, '**CHLORINATED WASTE**' and '**NON-CHLORINATED WASTE**'.
2). Containers may be empty glass bottles from solvents or poly jericans (10 L or less).
3) When in use they are to be placed inside a fume hood with the sash pulled down.
4) University Health and Safety Department will pick up the waste solvent on Friday. Label the bottle properly and sign it.

13.5.2 Silica

After solvent has evaporated, pour silica into a separate bottle. When the bottle is full label it. University Health and Safety will pick it up together with the waste solvent.

13.5.3 Teflon boiling chips

Place in waste container (i.e., beaker) under hood until solvent evaporates, then empty into trash can.

13.5.4 Glass

Place in 'Broken Glass Disposal Containers'. When containers are full, close according to directions on box; leave for janitors to pick-up or take out to the trash dumpster.

13.5.5 Foil

Place in trash can.

13.5.6 Fiberglass

Place in waste container (i.e., beaker) under hood until solvent evaporates, then empty into trash can.

13.5.7. XAD_2 and QF

Leave in soxhlet under hood until solvent has evaporated. Pour XAD_2 into container labeled '**USED XAD_2**'. Discard QF into trash can.

14.0 References

Following publications were consulted for the development of methods of PCB, pesticides and PAHs analysis in Air and Precipitation samples. Experimental procedure was modified according to our need.

Baker, J.E.; Eisenreich, S.J. PCBs and PAHs as Tracers of Particulate Dynamics in Large Lakes. J. Great Lakes Res., 1989, 15(1),84-103.

Bidleman, T.F.; Mathews, J.R.; Olney, C.E.; and Rice, C.P. Separation of Polychlorinated Biphenyl, Chlordane and p-p DDT from Toxaphene by silicic acid column chromatography. J. Ass. off. analyt. chem., 1978, 61, 820-828.

Hermanson, M.H. and Hites, R.A. Long-Term Measurement of Atmospheric Polychlorinated Biphenyls in the Vicinity of Superfund dumps. Environ. Sci. Technol., 1989, 23. No. 10, 1253-1258.

Marti, E.A. Armstrong D.E. Polychlorinated Biphenyls In Lake Michigan Tributaries. J. Great Lakes Res., 1990, 16(3): 396-405

Mc Veety, B.D. and Hites, R.A. Atmospheric Deposition of Polycyclic Aromatic Hydrocarbons to Water Surfaces: A Mass Balance Approach. Atmos. Environ., 1988, 22, 511-536.

Mullin, M.D. PCB Workshop, U.S. EPA Large Lakes Research Station, Grosse Ile. MI, June 1985.

Murphy, T. J. and Rzeszutko, C.P. Precipitation inputs of PCBs to Lake Michigan. J. Great Lakes Res., December 1977. Internat. Assoc. Great Lakes Res., 3(3-4): 305-312.

Swackhamer, D.L.; Mc Veety, B.D.; and Hites R.A. Deposition and Evaporation of Polychlorinated Biphenyl congeners to and from Siskiwit Lake, Isle Royale, Lake Superior. Environ, Sci. Technol., 1988, 22, 664-672.

Sweet, C.W.; Vermette, S.J.; and Gatz, D.F. Atmospheric Deposition of Toxic Materials: A Compound of the Green Bay Mass Balance Study. 1992, Contract Report 530, Illinois State Water Survey, Champaign, IL 61820.

Personal communication with:
Hites, R.A. and his group from Indiana University, 1990-1993
Eisenreich S.J. and his group, 1990-1993
Swackhamer, D.L., 1990-1993